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Award Number: DAMD17-02-1-0070

TITLE: Prevention of Post-Radiotherapy Failure in Prostate Cancer by Vitamin D

PRINCIPAL INVESTIGATOR: Srinivasan Vijayakumar, Ph.D.

CONTRACTING ORGANIZATION: University of California, Davis
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REPORT DATE: March 2006

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
March 2006	Annual	1 Mar 05 – 28 Feb 06

4. TITLE AND SUBTITLE

Prevention of Post-Radiotherapy Failure in Prostate Cancer by Vitamin D

5a. CONTRACT NUMBER

5b. GRANT NUMBER
DAMD17-02-1-0070

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of California, Davis
Davis, California 95616-8670

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Prostate cancer patients receive either surgery or radiation therapy as treatment for cancer. Among patients receiving radiation therapy, nearly 50% have an elevation of PSA within five years of treatment. These patients then receive hormone treatment. In this study, we wish to test the theory that chemopreventive agents, which shoe the ability to prevent or delay the growth of prostate cancer cells in the laboratory, may also prevent or delay the growth of prostate cancer cells in the laboratory, may also prevent or delay the reappearance of prostate cancer in patients who have undergone radiation to treat their prostate cancer. We propose to have prostate cancer patients who have undergone radiation treatment take a non-toxic chemopreventive agent [a synthetic form of vitamin D, 1 ˜-hydroxyvitamin D5] for two years and see if their reoccurrence rate can be decreased. Unlike regular vitamin D, D5 does not make calcium in the bloodstream and reach levels that cause serious side effects. Forty patients will participate. They will be randomized to D5 or placebo arms. A biopsy will be done at the end of the study and the tissue will be analyzed for any benefit of D5 in decreasing the recurrence of prostate cancer and also for any differences between the groups in terms of expressed intermediate molecular biomarkers.

15. Subject Terms (keywords previously assigned to proposal abstract or terms which apply to this award)
Radiation therapy, vitamin D analog, PSA, Biomarkers, D5, Prostate Cancer, chemoprevention

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
U	U	U	UU	38	**19b. TELEPHONE NUMBER** *(include area code)*

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I. INTRODUCTION

We plan to conduct a phase I/II safety/chemoprevention study to determine whether taking a non-toxic Vitamin D analog, 1α(OH)D5 (D5), can safely delay prostate cancer recurrence when administered after radiation therapy (RT). The newly synthesized analog 1α(OH)D5 (1α-Hydroxy-24-ethyl-cholecalciferol) has shown anti-tumor activity at non-hypercalcemic concentrations in animals. Based on our preliminary research, we believe D5 can be given in effective doses without causing harmful side effects. Forty randomized patients will receive either D5 or placebo, 12-60 months after completion of RT (20 patients/arm). During the study patients will be closely monitored for hypercalcemia as well as other potential toxicities. At the end of the study, subjects will receive final laboratory and clinical evaluations and undergo a prostate biopsy. Study endpoints include differences between study groups in drug tolerance and compliance, toxicity, quality of life, biomarker presence and proportion of patients developing PSA-based biochemical failure or clinical failure. Biopsies will be evaluated for selective markers indicating any benefit of D5 in decreasing the recurrence of prostate cancer and also for any differences between the groups in terms of expressed intermediate molecular biomarkers. Patients will continue to be followed for any clinical recurrences or toxicity as part of their usual cancer care.

II. BODY

2.1. The following are the tasks for this study:

Task		**Progress**
Task 1	Obtain necessary clinical trial approvals.	Done except FDA Approval
Task 2	Register patients to start the clinical study.	Not yet initiated
Task 3	Following up patients on study.	Not yet initiated
Task 4	Complete the clinical study.	Not yet initiated
Task 5	Follow up patients with Vitamin D treatments.	Not yet initiated

2.2. With regard to Task 1, following is work done and accomplishments

Date	**Progress**
February, 2004	Grant was officially transferred from the University of Illinois at Chicago (UIC) to the University of California, Davis (UCD), a necessary step in allowing us to conduct the study once we obtain IRB approval at UCD and DOD approval.
March, 2004	Completion of Clinical Protocol and Approval by UC Davis IRB. Our principal accomplishment during this period was finalizing the clinical protocol for the study with D5 and securing the approval, with pending minor revision, by the UC Davis IRB for the clinical trial (See Appendix 6 submitted with 2004 Annual Report**).** On March 8, 2004, the UC Davis

	IRB met and approved the protocol, pending minor revisions. Revisions (mostly wording) were done and the protocol to resubmitted to the IRB Committee Chair for final approval. The development of the clinical protocol began by taking into account the critique of the protocol made by the UIC Cancer Center Protocol Review Committee in July 2002. While at UIC, Dr. Vijayakumar brought the protocol to about 80% completion. He had set up an Executive Committee to prepare the protocol, and they met several times to design the study. (Minutes were submitted to the DOD previously). Further fine-tuning occurred at UC Davis. In 2003, Dr. Vijayakumar shared the protocol with UCD Radiation Oncology faculty at regular faculty meetings, seeking their input on how to improve the protocol and incorporating their suggestions. Attendees at these meetings were Radiation Oncologists Dr. Allan Chen, Dr. Rachel Chou, Dr. Zelanna Goldberg, Dr. Samir Narayan and Dr. Janice Ryu, and Physicists Dr. Julian Perks, Dr. Robin Stern, and Dr. Claus Yang. In addition, over several months in the fall of 2003, Dr. Vijayakumar consulted extensively with the statistician for the UCD Cancer Center, Dr. Laurel Beckett, to confirm and modify the study design. Dr. Vijayakumar also recruited other investigators for the protocol, especially clinical faculty who will be enrolling patients in the trial, and assembled the rest of his team for the study (Clinical Research Associates, consultants). In October 2003, Dr. Vijayakumar made a presentation to discuss the protocol with several UCD Cancer Center faculty. At the meeting was the director of the Cancer Center, Dr. Ralph deVere White (Urology), as well as Dr. Samir Narayan (Radiation Oncology), Dr. Paul Gummerlock (Hematology & Oncology), Dr. Rajendra Mehta—via speaker phone (Surgical Oncology, UIC), Dr. William Hall (Radiation Oncology), and Phil Boerner (Writer, Radiation Oncology). As a result of this meeting, several important modifications were made to the protocol, including adjusting eligibility criteria, study endpoints, and having a data and safety monitoring committee review the study periodically once it commences.
November, 2003	Before submitting the updated protocol to the UC Davis Cancer Center Scientific Review Committee, Dr. Vijayakumar wanted to have input from the DOD's pre-review. Dr. Vijayakumar received the DOD pre-review of the Vitamin D5 study, and incorporated the valuable suggestions made there into the protocol.
December 2003	Dr. Vijayakumar made a presentation to the UCD Cancer Center Scientific Review Committee and subsequently this committee approved the D5 protocol (see Appendices 1 and 2 submitted with 2004 Annual Report). (This committee's approval is required prior to submitting a protocol t the UCD IRB.) On the advice of this committee, we added a

	“Treatment Plan” section to the protocol.
February 19, 2004	The D5 protocol was submitted to the UC Davis IRB (the D5 protocol was submitted to the UC Davis IRB (see Appendix 5 submitted with 2004 Annual Report). The protocol was approved, pending minor revision, on March 8, 2004. When we make the minor revision and obtain final IRB approval, we will submit the protocol to the DOD for approval.
October 26, 2004	Updated our Statement of Work (SOW) (see Appendix 1 submitted with 2005 Annual Report).
November 4, 2004	Since the process of required approvals is taking longer than expected, we requested and received a no-cost extension from the DOD for the study, to February 2006 (see Appendix 2 submitted with 2005 Annual Report).
December 6, 2004	Obtained DOD approval for the study (see Appendix 3 submitted with 2005 Annual Report).
December 15, 2004	Obtained UC Davis IRB re-approval for the study, accepting the DOD's changes (see Appendix 4 submitted with 2005 Annual Report).
February 22, 2005	Requested annual renewal of this study with our IRB (see Appendix 5 submitted with 2005 Annual Report).
September 2005-January 2006	Please note Appendix 1, 2006 Annual Report to view papers/publications resulting from scholarly work of Dr. Vijayakumar and his colleagues.
January, 2006	FDA is requiring repeat stability testing of study drug. An India-based company named SaidruSyn has been contracted to do this. This company has a great deal of experience working with the FDA (see Appendix 2, 2006 Annual Report).
February 8, 2006	No-Cost Extension requested (see Appendix 2, 2006 Annual Report).
February 28, 2006	Additional information E-mailed to Wendy Baker to attach to No Cost Extension Request (see Appendix 1, 2006 Annual Report).
March 9, 2006	No-Cost Extension approved for one year. Amendment/Modification #P00004 attached as Appendix 3, 2006 Annual Report.

We are aggressively pursuing FDA approval for the study drug, which we believe will be received during the year 2006/07. Stability testing on the pill is currently being conducted.

III. KEY RESEARCH ACCOMPLISHMENTS

As this is a clinical study, only key findings generated from this clinical study can be considered as key research accomplishments. Since the clinical trial has not even begun and is pending approval by the FDA. We have not started the clinical trial, however, we did accomplish the following in the area of Vitamin D analogs/D5’s are in cancer/cancer presentations.

Laboratory studies:

Summary

Vitamin D3 (Calcitriol) has been used both alone and in combination with chemotherapeutic agents such as Docetaxel to suppress the growth of prostate tumors. However vitamin D3 has also been shown to upregulate the levels of androgen receptor in prostate tumor cells in culture and in addition has been linked to dose-limiting hypercalcemia. Here we confirm those data indicating that 0.1μM vitamin D3 substantially increases the expression of androgen receptor protein, starting 4 days after vitamin treatment. This increase in androgen receptor was linked to a similar increase in PSA. Vitamin D5 reportedly exhibits reduced hypercalcemia in animal models making it a more attractive molecule for therapeutic use. Using doses of vitamin D3 and D5 that were equivalently cytostatic, as determined by an MTT assay, vitamin D5 showed a consistently reduced ability to activate both the androgen receptor and its down stream target, PSA. This indicates that vitamin D5 presents a more useful profile of biological activities for studies tracking prostate growth using PSA as a surrogate marker.

Methods

MTT Assay LNCaP cells were plated in 24-well tissue culture plates at 2 x 10^4/well. Cells were allowed to attach overnight and then treated with either control media (RPMI/5% FCS/0.1% Penicillin/Streptomycin), control media supplemented with vitamin D3 (100nM), or control media supplemented with vitamin D5 (10nM – 2μM). Media was refreshed every 72 hours. At designated time points, dimethylthiazolyl-2, 5-diphenyltetrazolium bromide (MTT) was added to the culture supernatant and plates incubated for an additional one hour. Cells were then solubilized with DMSO and absorbance assessed as a measure of MTT uptake.

Western analysis LNCaP cells were plated at 2.5 x 106 cells/dish in 60mm tissue culture dishes and allowed to attach overnight. Cells were then treated with either, control media (RPMI/5% FCS/0.1% Penicillin/Streptomycin), control media supplemented with vitamin D3 (100nM), or control media supplemented with vitamin D5 (10nM – 2μM). At designated time points, whole cell lysates were collected and protein concentration determined using the Coomassie Plus Protein Assay (Pierce) following manufacturer's instructions. An equal amount of total protein per lane was fractionated by electrophoresis on either a 10% (PSA) or 4-15% (androgen receptor) SDS-polyacrylamide gel. Subsequent to electrophoresis, gels were transferred to a nitrocellulose membrane and immunoblotting was performed using either anti-PSA, anti-AR or anti-actin, and secondary antibodies coupled to horseradish peroxidase. Blots were developed using Pierce West Pico Chemiluminescent blot detection reagent according to manufacturer's instructions and exposed to film.

Results

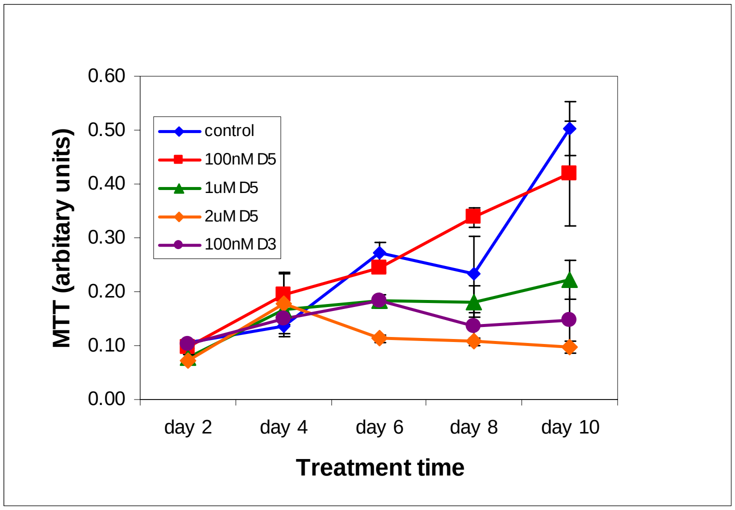

Figure. 1 Anti-proliferative effect of Vitamin D3 and D5. LNCaP prostate cancer cells were exposed to a range (10nM -2μM) of Vitamin D5 or 100nM Vitamin D3 for the times shown. Concentrations of Vitamin D5 between 1-2 μM were found to have an equivalent cytostatic effect as 100 nM Vitamin D3 (other Vitamin D5 concentrations not shown). Thus 1-2μM vitamin D5 and 0.1 μM vitamin D3 were considered of equivalent cytostatic potential.

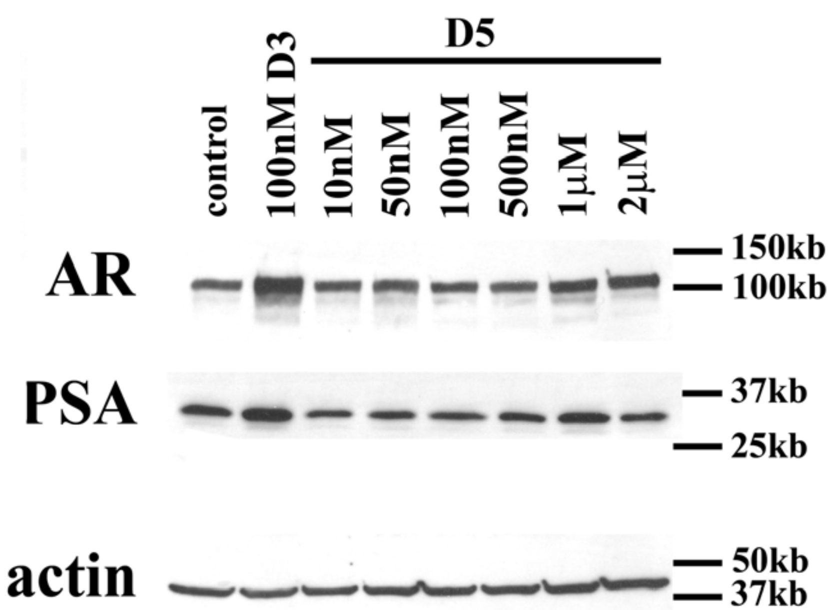

Figure 2. Androgen Receptor (AR) and PSA protein expression Levels of both androgen receptor and PSA were determined in the LNCaP prostate cancer cell line four days after treatment with Vitamins D3 or D5, at the concentrations shown. At vitamin concentrations that were equally cytostatic, Vitamin D3 treatment was linked to upregulation of both the androgen receptor and its transcriptionally regulated target, PSA

while cytostatically equivalent concentrations of Vitamin D5 showed minimal effect on the proteins studied.

IV. REPORTABLE OUTCOMES

See Section 2.2.

V. CONCLUSIONS

We have not initiated the clinical trial on this project. We still await FDA approval for the study drug. However, a number of accomplishments have been achieved (see Appendix 1).

VI. REFERENCES

Please see Appendix 7 for a copy of the following paper, regarding this study, and published during the past year:

Packianathan S, Mehta RG, Mehta RR, Hall WH, Boerner PS, Beckett LA, Vijayakumar S. Designing a randomized phase I/II prostate cancer chemoprevention trial using 1alpha-hydroxy- 24-ethyl-cholecalciferol, an analogue of vitamin D3. Cancer J. 2004;10(6):357-67.

A copy of the updated version of the protocol is submitted as Appendix 8, 2005 Annual Report.

VII. APPENDICES

1. Papers/publications resulting from scholarly work of Dr. Vijayakumar and his colleagues
2. No-Cost Extension Request, dated February 8, 2006
3. Amendment/Modification #P00004, dated March 9, 2006

APPENDIX 1

Lisa Worland/SOM/HS/UCD

02/28/2006 10:40 AM

To:

"Baker, Wendy A Ms USAMRAA" <wendy.cockerham@us.army.mil>

cc:

"Mishra, Nrusingha C Dr USAMRMC" <nrusingha.mishra@us.army.mil>, srinivasan.vijayakumar@ucdmc.ucdavis.edu, pnoble@ucdavis.edu, Megan Tilghman/SOM/HS/UCD@UCDavis, marie.rodriguez@ucdmc.ucdavis.edu

Subject:

Add'l Information for No-Cost Extension Request, #DAMD17-02-1-0070, PI: Vijayakumar

Dear Wendy,

Dr. Vijayakumar has asked that I forward you the following information to be included with our no-cost extension request. Please contact Dr. Vijayakumar (916) 734-7888 or myself (916) 734-8241 if you have any questions.

Thank you,

Lisa Worland

Please note the following papers and publications resulted from the scholarly work of Dr. Vijayakumar and his colleagues, although less than $15,000 was spent to date.

Dr. Vijayakumar was also invited to a Conference on Vitamin D Receptors Investigations: **Invited lecture-Prostate Cancer Clinical Trials with Vitamin D, CeDAR Symposium, Boston, MA, Sept., 2005.**

I. Peer Reviewed Papers:

1. Publications # 134 in Dr. Vijayakumar's CV. 2005 Vijayakumar, S., Mehta, R.R., Boerner, P.S., Packianathan, S. & Mehta, R.G. Clinical trials
involving vitamin D analogs in prostate cancer. Cancer Journal, 11(5): 362-73.

2. Publication # 129 in Dr. Vijayakumar's CV. 2004 Packianathan S, Mehta R, Mehta R, Hall W, Boerner P, Beckett L, Vijayakumar S.
Designing a randomized Phase I/II Prostate Cancer Chemoprevention Trial Using 1a hydroxy-24-ethyl-cholecalciferol, an Analog of Vitamin D3. The Cancer Journal, 10(6): 357-367.

3. One more paper is pending a decision from the Cancer Journal [see attached word document, item IV]

II. Scientific Abstracts:

Abstract # 109 in Dr. Vijayakumar's CV 108. 2004 Vijayakumar S, Mehta R, Mehta R, Hall W, Boerner P, Beckett L. "Clinical Trial Design in
Chemoprevention Studies: Using a Vitamin D5 Analog Study as an Example" American Radium Society 86th Annual Meeting, May 1-5, Napa Valley, California. (poster presentation)

Abstract # 111 in Dr. Vijayakumar's CV 108. 2005 Packianathan S, Vijayakumar S. "Intermediate Biomarkers in Male Genitourinary Cancers --
Penile Cancer." American Radium Society 87th Annual Meeting, April 30-May 4, Barcelona, Spain (poster presentation).

Abstract # 105 Babbar D., Gandhi M., Mehta R.G., Vijayakumar S. and Mehta R.R. Effects of 1a-hydroxyvitamin D5 on prostate cancer cells. Proc. Am. Assoc. Cancer Res. 44: 1269, 2003.

III. Abstract accepted for 2006 AACR Meeting

January 2006

2006 AACR Annual Meeting in Washington, DC

Title: The low-calcemic vitamin D analog 1-alpha-hydroxyvitamin D5 is anti-proliferative and does not increase androgen receptor expression in prostate cancer cells

Session ID: Cellular and Molecular Biology 17
Session Date and Start Time: Sunday, April 2, 2006 1:00 PM
Permanent Abstract Number: 931

GA Loredo1,2, XH Lu1,2, R Mehta3, S Vijayakumar2, ATM Vaughan1,2, and PM Ghosh1,2,4
1Sacramento VA Medical Center, Mather, CA; 2University of California Davis Medical Center, Sacramento, CA; 3University of Illinois, Chicago, IL ; 4University of Texas Health Science Center, San Antonio, TX

The active metabolite of vitamin D, calcitriol, is well established as an effective tumor suppressing agent that regulates cell growth and differentiation. However, its anti-tumor activity is achieved at doses that are hypercalcemic *in vivo.* In addition, it causes upregulation of androgen receptor (AR) expression in LNCaP cells, a transcription factor that induces the expression of androgen-responsive genes like prostate specific antigen (PSA). Prostate cancer is usually detected initially by rising PSA levels in the serum and PSA is considered a biological marker for monitoring the disease. Hence, increased AR expression, and therefore, increasing PSA levels by calcitrol are further deterrents to its use in prostate cancer. Therefore, a vitamin D3 analog, 1alpha-hydroxy-24-ethyl-cholecalciferol (1alpha[OH]D5), which in animal studies has been demonstrated not to alter calcium regulation, was evaluated in prostate cancer cell lines. After exposure of the cancer cells to 1alpha[OH]D5, its effect on proliferation was assessed using the dimethylthiazolyl-2,5-diphenyltetrazolium bromide (MTT) assay. In parallel experiments, the effect on AR expression was measured by immunoblotting whole cell lysates of LNCaP cells with an anti-AR antibody. Compared to calcitriol, 1alpha[OH]D5 was more effective in reducing growth rates of the androgen-dependent prostate cancer cell line LNCaP, but similar to calcitriol had no significant effect on androgen-independent clones of LNCaP or DU145 cells. However, unlike calcitriol, 1alpha[OH]D5 did not cause an increase in AR expression, suggesting distinct mechanisms of action between these two vitamin D receptor ligands. Taken together with the previously demonstrated low-calcemic character of 1alpha[OH]D5 *in vivo*, these results indicate the significant potential of 1alpha[OH]D5 as a more suitable drug for use in prostate cancer.

IV. Paper submitted to the Cancer Journal (see Word Document attached)

D5BreastReview FINAL.doc
* *

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Clinical Trials with Chemopreventive Agents for the Treatment of Breast Cancer

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Rajeshwari R. Mehta, Ph.D., Associate Professor[2],
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Supported in part by DOD Grant No: DAMD17-02-1-0070, HSRRB Log No. A-11241 to SV.

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Key words: breast cancer, vitamin D, chemoprevention
Running Title: Breast Cancer Chemopreventive Clinical Trials

Abstract

This article comprehensively reviews the clinical trials and considers the future directions of the use of vitamin D and its analogs in the treatment or chemoprevention of breast cancer. Chemopreventive treatment strategies strive to delay the onset of certain cancers or prevent the progression of malignant disease after diagnosis or delay the advent of recurrence after curative treatment. We first summarize the epidemiological evidence that led to the hypothesis that vitamin D may have an anti-cancer activity. Vitamin D shows great potential as a therapy for breast cancer. However, its use in clinical trials has been hindered by the induction of hypercalcemia at a concentration required to suppress cancer cell proliferation. This has led to the development of less calcemic analogs of vitamin D. We review the clinical trials with breast cancer patients using vitamin D analogs, concluding with our study with $1\alpha(OH)D_5$, which will start shortly.

Search strategy and selection criteria

Data for this review were identified by searches of PubMed, the Cochrane Library, Biosis, and references from relevant articles, using the search terms “vitamin D”, “breast cancer”, “chemoprevention”, and “vitamin D analog”. Abstracts from recent international meetings were also reviewed but were included only when they were the only known reference to the clinical trial or the research mentioned. Only papers published in English were included.

Introduction

Breast cancer, the strongest risk factors for which include gender, age, and country of birth, continues to be significant source of morbidity and mortality for women. Other primary risk factors for breast cancer are related to the female reproductive cycle, and include age at menarche, nulliparity, age at first birth and duration of lactation, and age at menopause. Additional risk factors include exogenous estrogens, radiation, alcohol consumption, and higher income and educational level [1]. Interestingly, location of residence has also been cited as a risk factor for breast cancer, which combines the two previously cited risk factors of radiation and country of birth [2]. In the United States, the American Cancer Society estimates that 211,240 women are likely to be diagnosed with breast cancer in 2005 and 40,410 will die from their disease, making it the cancer with the greatest incidence in the United States and the second highest mortality, after lung cancer [3].

Chemoprevention is an intervention in the carcinogenic process, possibly by a synthetic compound, which blocks, arrests, or reverses the progression of cancer [4, 5]. Age is the most significant risk factor for many cancers, and awareness of this fact is a driving force behind research in cancer chemoprevention. With life expectancy continuing to rise in the general population, the incidence of breast cancer is likely to increase in the coming years. A large proportion of women diagnosed with this disease can expect to experience significant morbidity during the course of their illness and the associated treatments. Chemopreventive treatment strategies strive to delay the onset of certain cancers or prevent the progression of malignant disease after diagnosis or delay the advent of recurrence after curative treatment. Initiatives using safe chemopreventive agents that are directed toward these tasks would be greatly welcome and are likely to have a major impact on women's health. Initial patient recruitment for chemoprevention trials, however, is likely to be focused on patient groups with the specific high-risk factors alluded to earlier.

One potential chemopreventive agent for breast cancer that is currently being developed at our institutions is $1\alpha(OH)D_5$, or vitamin D5, a synthetic analog of vitamin D. The effects of this analog will soon be investigated in two clinical trials, one involving breast cancer patients and the other with prostate cancer patients [6]. Vitamin D deficiency is common in the elderly [7]. Aging lowers the ultraviolet radiation-mediated production of cholecalciferol in the skin. Moreover, estrogen deficiency, which primarily affects postmenopausal women, decreases the metabolic activation of vitamin D, as well as the expression of the vitamin D receptor (VDR) [8]. VDRs are known to be expressed in a variety of cancer cells. Specific VDR polymorphisms can increase susceptibility to breast cancer and women with certain genotypic variations may also be burdened with a more aggressive form of the disease, especially if the cancer metastasizes [9]. In addition, deficiency in vitamin D *per se* may contribute to the incidence and mortality of breast cancer (see below), and its prevention may be thus be possible through increased sunlight exposure, improved diet, and supplemental vitamin D. Several studies measuring solar radiation have supported its beneficial role in breast and other cancers through its mediation of vitamin D synthesis, providing support for the hypothesis that vitamin D may provide some degree of protection against cancer. Epidemiologists estimates that perhaps 30-60% of all cancers could be avoided by modifications in diet [10], and vitamin D is ingested in the diet, as well as synthesized through skin exposure to solar radiation.

Vitamin D

Vitamin D was discovered by Edward Mellanby in 1919 in his experiments using dogs that were exclusively raised indoors, without exposure to sunlight or ultraviolet light [11]. Subsequently, E.V. McCollum was able to differentiate between vitamin A and vitamin D [12], both fat soluble vitamins. Vitamin D is a steroid hormone that has been shown to have antiproliferative and anti-tumor properties, making it a strong candidate for chemoprevention in breast or other malignancies. However, the usefulness of vitamin D in pharmacologic doses or over long periods of time has been limited because it can cause life-threatening hypercalcemia. For this reason, many new analogs that demonstrate less calcemic activity than vitamin D have been developed and some of these are

being tested in phase I and phase II trials. Several recent reviews have also addressed the anti-cancer effects of vitamin D on breast cancer cells [13, 14].

A recent paper by Bertone-Johnson et al. suggested that vitamin D may be modestly beneficial for management of breast disease [15]. The researchers examined the relationship between stored plasma levels of 25-hydroxyvitamin D [25(OH)D] and 1,25-dihydroxyvitamin D [1,25(OH)2D] and risk of breast cancer in a case-control study nested within the Nurses' Health Study cohort. Breast cancer cases had a lower mean 25(OH)D level than controls. The association was stronger in women 60 years and older.

Vitamin D and Cancer Risk

That adequate vitamin D intake may prevent the development of certain diseases—such as rickets, osteoporosis, and tuberculosis—and even specific types of cancer, has been well documented [16, 17, 18, 19, 20]. The initial evidence suggesting an association between vitamin D and cancer protection was primarily epidemiologic in nature. Peller, for instance, observed that in occupations and environments wherein skin cancer rates were higher, the rates for other cancers were lower [21]. Subsequently, Apperly also reported that populations living farther from the equator had higher overall cancer death rates compared to those living closer to the equator, suggesting that increased sun exposure—and with it increased synthesis of vitamin D—led to decreased cancer-associated mortality [22].

Historically, breast cancer mortality rates among American women have varied geographically and longitudinally, with the highest mortality occurring in the Northeast and the lowest mortality being reported in the South (2, 19, 23, 24], suggesting that solar radiation, which leads to vitamin D synthesis, might be protective against breast cancer [25]. Breast cancer mortality is also increased in cities compared to rural areas [2], apparently because people living in urban areas may receive less sunlight exposure than those in rural areas at the same latitude, owing to air pollution. For instance, an analysis of data from a national cohort NHANES I Epidemiologic Follow-up Study found that among women living in areas of high solar radiation, sunlight exposure and adequate dietary vitamin D

intake were associated with a 25-65% reduction in breast cancer risk [24]. Gorham *et al*. too have showed statistically significant positive associations between acid haze air pollution, which blocks ultraviolet-B light, and age-adjusted breast and colon cancer mortality rates in a study covering 20 Canadian cities [17]. They hypothesized that the populations in such cities with high levels of acid haze may have been encumbered with vitamin D deficiencies. In addition, a similar ecological study in the former USSR by Gorham *et al*. also found a pattern of increased breast cancer incidence in those regions experiencing low sunlight levels [26].

These geographic variations in which breast cancer mortality is inversely proportional to the intensity of the local sunlight, have also been duplicated in the United States [19]. More recent studies have found that exposure to sunlight was inversely associated with mortality from breast cancer [27], as was UV-BH radiation exposure *per se* [28]. Other investigations have also suggested this epidemiologic link between vitamin D and breast cancer [29, 30, 31]. The most likely mechanism by which sunlight exposure could inhibit the development of breast cancer is through the production of vitamin D. Casual exposure to sunlight remains one of the primary sources of vitamin D for women in the U.S., which, along with diet, fortunately is a modifiable lifestyle factor.

A few studies contradict these findings. For example, Hiatt et al. identified no relationship between elevated prediagnostic serum levels of $1,25(OH)_2D$ and the later diagnosis of breast cancer. However, the serum levels of vitamin D in this study were obtained an average of 15 years prior to the actual diagnosis of cancer. This, therefore, left unanswered the possibility that elevated vitamin D could have a protective effect at a time closer to the clinically evident breast cancer [32].

A single Canadian case control study evaluating dietary histories also did not identify an association between low vitamin D consumption and breast cancer development in women [33]. Indeed, breast cancer patients were found to have had a higher consumption of vitamin D than comparable controls. This study, however, did not consider the sunlight exposure-induced synthesis of vitamin D in these subjects.

Another study, examining incidence of breast cancer rather than mortality, also found little evidence of regional variation in breast cancer incidence rates [34]. Sturgeon *et al*, however, have recently argued that the historically higher breast cancer mortality rates reported in the North are in decline. Women in the Northeast are now experiencing a faster rate of decline in breast cancer mortality than their counterparts in the South, especially in specific groups such as black women of all ages and white women aged 20-49 years [23].

Likewise, a study in Norway did not identify a negative association between cancer incidence and mortality and geographical latitude [35]. However, those investigators did point out that cases of breast, colon, and prostate cancer diagnosed in the summer and fall -- the seasons when serum levels of vitamin D_3 are expected to be the highest -- had a significantly better prognosis relative to the cases diagnosed during the winter months. Thus, vitamin D may have a beneficial effect on cancer specific mortality and supplemental vitamin D intake may improve cancer-related outcomes.

Clinical Trials with Vitamin D or its Analogs

There have been only a few breast cancer clinical trials with vitamin D or one of its analogs; these are reported in Table 1. In contrast to prostate cancer, such investigations in clinical trials are not as advanced (see Vijayakumar et al. for a summary of clinical trials with prostate cancer patients and vitamin D analogs [36]).

To the best of our knowledge, the first study involved the use of topically applied calcipotriol. This vitamin D analog, also known as compound MC903, was used in the treatment of advanced breast cancer [37]. Treatment was administered to 19 patients with locally advanced or cutaneous metastatic breast cancer, with selected cancer nodules receiving the topically applied calcipotriol in doses of 100 micrograms daily. Five patients had to be withdrawn from the study before completion of the treatment; two of them because they developed hypercalcemia. The response rate too was low, with improvements noted in only 3 of the 14 patients who completed the 6 weeks of

treatment (these 3 showed a 50% reduction in the bidimensional diameter of treated lesions). Of the remaining 14 patients, 5 unfortunately experienced progression of their disease, 5 reportedly had no change in their disease, and one had only a minimal response. Vitamin D receptors (VDR) were identifiable in the breast cancer cells of 7 patients, including all 4 who had had some response to the topical treatment. These data with calcipotriol suggested that this vitamin D analog may function through a mechanism involving the VDR.

Gulliford *et al* conducted a phase I trial to evaluate the maximum tolerated dose of another vitamin D analog, EB 1089 (Seocalcitol), in 36 patients with advanced breast (n=25) or colorectal (n=11) cancers. EB 1089 is a newly synthesized vitamin D analog that is much more potent in regulating cell growth and differentiation than cholecalciferol ($1\alpha,25(OH)_2D_3$), has a lower tendency to induce hypercalcemia, and can induce apoptosis in some types of cancer cells [38]. All patients received the EB 1089 solution for 5 consecutive days per protocol and it was continued as compassionate treatment beyond that time in 21 cases for 10-234 days. The first 11 patients enrolled had also received a single dose one week before starting the schedule of protocol doses. The treatment doses used started at 0.15 $\mu g/m^2$ body surface area daily and was gradually increased to a maximum of 17.0 $\mu g/m^2$ daily.

All patients receiving the maximum dose suffered from hypercalcemic toxicity. This study identified the optimal dose of EB 1089 to be 7.0 $\mu g/m^2$ daily. Six of the patients receiving compassionate treatment for more than 90 days showed stabilization of their disease. EB 1089 was found to be much less calcemic than $1\alpha,25(OH)_2D_3$. Eleven patients in the protocol treatment phase experienced hypercalcemia, with 4 showing severe hypercalcemia at doses of 0.45, 12.4 and 17 $\mu g/m^2$. During the compassionate treatment phase, 10 patients experienced hypercalcemia, six of them severely. However, this study did not demonstrate any anti-tumor effect, as determined by an objective reduction in tumor volume, although six patients showed stabilization of their disease for over three months. Clinical trials evaluating the effectiveness of EB 1089 was then carried out in other cancer types as well [38].

The Women's Health Initiative (WHI) Clinical Trial and Observational Study also includes a vitamin D supplementation arm. Supplementation was primarily hypothesized to prevent hip and other fractures and secondarily prevent colorectal and breast cancer [39]. The WHI was established by the National Institutes of Health (NIH) in 1991 and the study involves over 161,000 postmenopausal women aged 50-79, who were enrolled in the study at 40 nation-wide clinical centers between 1993 and 1998.

As indicated, one of the hypotheses being tested in the vitamin D arm of the WHI study is that women who receive calcium and vitamin D supplements will benefit with a lower risk of breast cancer than women receiving a placebo. This large-scale trial of a breast cancer chemopreventive agent is a 1:1 randomized double-blind trial using 1000 mg elemental calcium plus 400 international units (IU) of vitamin D_3 daily, versus a placebo. Participants take two pills per day. The planned completion date of the WHI study is 2007 and it is projected to enroll 45,000 women in the calcium and vitamin D supplementation arm. The findings of this study are eagerly awaited.

Vitamin D_5

The first evaluation of D_5 as a chemopreventive agent for breast cancer will be conducted in our upcoming clinical trial. At the University of Illinois at Chicago (UIC) we have carefully designed a combined Phase I/II clinical trial to evaluate the safety and efficacy of $1\alpha(OH)D_5$ in patients with metastatic breast cancer. This safety/chemoprevention study, in addition to finding the maximum tolerated dose (MTD) for D_5, will monitor the clinical response as measured by decreases in measurable disease determined by physical examination, radiographic studies, and/or nuclear medicine scans. Forty-two breast cancer patients who have received conventional treatment but not responded well will receive D_5, beginning at least four weeks after the completion of their prior therapy. Patients will receive pre-treatment screening and baseline evaluations, including serum chemistries, urinalysis, chest x-ray, electrocardiogram, renal ultrasound, and bone scan. Once they start the trial, subjects will be monitored in the clinic every week the first four weeks and then every three weeks for the remainder of the study. Patients will be evaluated for bone pain and possible adverse events and have complete blood count (CBC), differential, and platelets at every

study visit, along with serum chemistries. Appropriate radiographic and nuclear medicine imaging studies will be performed at week 12 and week 28, or sooner if clinical examination is suspicious for disease progression. Patients will also receive additional evaluations up to six months after the conclusion of taking D5 for the study.

The breast cancer trial with D_5 at UIC is a companion trial to another that will soon be conducted with D_5 and prostate cancer patients [6]. There are many similarities between breast and prostate cancer, which both respond to vitamin D. That trial will also be a phase I/II safety/chemoprevention study to determine whether $1\alpha(OH)D_5$ can safely delay prostate cancer recurrence when administered after radiation therapy (RT). Because of its low toxicity, D_5 can be studied in healthy volunteers. Table 2 compares our two planned trials with D_5. Forty randomized patients will receive either $1\alpha(OH)D_5$ or placebo, beginning 12-60 months after completion of RT. In contrast to earlier studies with other vitamin D analogs [40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53], this study includes a placebo-controlled arm for comparison, as well as a one-month run-in period. Patients will receive baseline clinical staging, pre-treatment biopsy and serum PSA levels. In the prostate cancer/D_5 study to be conducted at UC Davis, subjects will be monitored using serum chemistries and albumin weekly in the first month. Individuals with stable calcium levels will then have weekly phone calls and monthly clinical assessments. Serum chemistries, albumin and PTH, and urine electrolytes will be obtained monthly. PTH will be monitored biannually. Individuals with stable calcium levels at four months will transition to a four-month monitoring cycle, with chemistries, albumin and PTH, and urine electrolytes drawn immediately prior to a visit. At the end of the study, subjects will receive final laboratory and clinical evaluations and undergo a prostate biopsy. Patients will receive two years of post-treatment follow-up. It is important to establish biomarkers to determine if chemopreventive agents are being effective against cancer, something this study addresses in that it will be seeking intermediate biomarkers for prostate cancer.

In addition to epidemiologic and ecological studies, many animal studies have pointed to the possibility that vitamin D may be an effective chemopreventive agent against breast cancer. There are a number of good reviews on these

topics [54, 55, 56, 57]. These animal studies are the first steps in the process that a new chemopreventive agent must undergo, which includes preclinical studies in *in vitro* and *in vivo* animal experiments, followed by phase I, II, and III clinical trials for toxicity and efficacy.

Conclusion

Further studies are needed to find ways to reduce the side effects of chemopreventive agents and investigators must use extreme care in selecting women for chemopreventive studies relating to breast cancer. Investigators are still discerning the overall risk to benefit ratio in the context of chemoprevention of cancer in healthy subjects. All clinical studies must undertake a full assessment of side effects of their study drugs, for if the side effects put women at great risk, then the chemopreventive is a failure. There is a trade-off in prescribing preventive drugs in healthy patients. Additional clinical trials will discern whether the benefits of vitamin D analogs in preventing breast cancer are large while the harm is small. Vitamin D5 is one of many novel agents that will be tested in upcoming clinical trials. The National Cancer Institute, American Cancer Society, and other funders need to expand chemoprevention research to deepen our understanding and create a dramatic lowering of cancer incidence and mortality rates. Through the process of preclinical and clinical studies, effective chemopreventive agents will be identified to prevent breast cancer.

Table 1. Studies with Breast Cancer Patients and Vitamin D Analog Therapy					
Author / P.I.	**# patients**	**Year Pub.**	**Therapy**	**Dose / frequency**	**Duration**
Bower [37]	19	1991	calcipotriol ointment	100 µg, QD	6 weeks
Gulliford [58]	36	1998	EB 1089	0.15 to 17.0 µg/m^2 QD	1.5-33.5 weeks (10 - 234 days)
The Women's Health Initiative Study Group [39]	45,000 women without breast cancer	1998	calcium + vitamin D_3	1000 mg elemental calcium + 400 international units vitamin D_3 QD	8 years (to be completed in 2007)
Das Gupta & Salti (planned study)	42	2006	D5	5-35 µg, QD	12 weeks

/m^2 = per meter squared of body surface area
QD = daily
µg = micrograms

Table 2. Comparison of Planned Studies with the Vitamin D Analog, D5		
Study Criteria	**Breast Cancer**	**Prostate Cancer**
Cancer stage	metastatic cancer (except brain metastases); must have failed treatment	high risk, non-metastatic cancer; post radiation therapy with curative intention
Number of subjects	42 subjects	40 subjects
Duration of treatment	12 weeks; follow-up blood tests to 28 weeks; then every 2 months for 6 months or until death	2 years; follow-up testing as long as possible during regular cancer care visits
Study type/goals	Phase I/II combined/toxicity & efficacy	Phase I/II combined/toxicity & efficacy; also intermediate biomarker response-seeking study
Subjects gender	females	males
Measured response(s) to treatment	Complete disappearance of all tumor masses; normalization of all laboratory parameters; no new lesions; resolution of all symptoms related to cancer	PSA does not rise (failure is 3 consecutive increases in PSA); no PSA failure [59]; no cancer in end-of-study biopsy specimens; no toxicity.
Partial Response to Treatment	A >50% decrease in the sum of the products of the diameters of any measurable lesions; recalcification of ≥50% of osteolytic lesion; reduction of >50% in the number of areas of increased uptake on bone scan; measures for stable disease, progressive disease, and recurrence included.	Drug discontinuation or dose reduction required; quality of life decline; differences in biomarkers profile.
Design	Single arm; dose-escalation from 5-35 µg, QD; 7 cohorts of 6 subjects each will take different doses of D_5; not randomized	Double arm (20 subjects receive study drug; 20 receive placebo); 10 µg, QD dose (no dose escalation); double-blinded, randomized
Duration of Study	90+ weeks	2 years
Check-up evaluations frequency	Patients will be followed in clinic every week for the first 4 weeks & then every 3 weeks	Patients will be seen once a week for the first month; then seen once a month, with weekly phone calls by the CRA; then every 4 months, with monthly phone calls.
Check-up evaluations	Initial physical examination, including pain evaluation, hematology, urinalysis, serum chemistries, CXR, EKG, CT scans, renal ultrasound, and bone scan. Monthly from weeks 12 to 28: blood tests, bone pain evaluations, adverse effects evaluations, hematology, and serum chemistries. Radiographic & nuclear imaging studies at weeks 12 and 28.	Initial physical examination, DRE & blood tests; Weekly interviews with the CRA and weekly evaluations of calcium and phosphorus in blood, albumin, Chem 7, and urine electrolytes; PTH at baseline and every 4 months; end of study biopsy

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February 8, 2006

Wendy Baker
Contract Specialist
U.S. Army Medical Research Acquisition Activity
820 Chandler Street
Ft. Detrick, MD 21702-5014

RE: Project: Prevention of Post-Radiotherapy Failure in Prostate Cancer by Vitamin D
Award #DAMD17-02-1-0070, P00002, Performance Period: March 1, 2002-March 31, 2006

Dear Ms. Baker:

We would like to request a no-cost extension of the grant period for our vitamin D5 Study, with the new grant period ending March 31, 2008. We are still awaiting FDA approval for Protocol: *"A Phase I/II Double-Blinded, Randomized Clinical Trial to Prevent/Delay Biochemical and Clinical Failure in High-Risk, Non-Metastatic Prostate Cancer Patients After Radiotherapy, Using I a-Hydroxyvitamin D5 Versus Placebo: A Tolerance-Finding and Intermediate Biomarker Response-Seeking Study"*. As indicated in our previous request, the grant funds are not currently being spent even though the University of California, Davis and the University of Illinois (subcontractor) have devoted considerable time on this project working to obtain FDA approval. We wish to save the grant funds for the actual Clinical Trial.

As indicated in the enclosed E-mail from Professor Mehta, University of Illinois, regarding the status of FDA approval for the study drug, D5, the FDA has told us to repeat the stability testing. The studies will be done by a company called SaidruSyn. This is an India-based company that had synthesized D5 for our clinical studies. They have a great deal of experience working with the FDA. I am hopeful that we will be able to get approval soon. The capsules have been prepared for the entire stability studies and have been shipped to SaidruSyn. I apologize for the delay in initiating studies with prostate cancer but it clearly depends on our getting approval from the FDA. If you have any specific questions, please do not hesitate to contact me at (916) 734-8252.

Sincerely,

Srinivasan Vijayakumar, M.D.
Professor and Chair
Department of Radiation Oncology

SV:lw

Enclosure

----- Forwarded by Philip Boerner/SOM/HS/UCD on 02/08/2006 12:03 PM -----

Mehta Rajendra <rmehta@iitri.org>

01/25/2006 08:49 AM

To: '"svijayakumar@aol.com"' <svijayakumar@aol.com>, vijay@ucdavis.edu, Philip Boerner <philip.boerner@ucdmc.ucdavis.edu>

cc:

Subject: RE: [Fwd: Re: Grant Close Out]

Dear Dr. Vijayakumar:

I am sorry for the delay in my response. As you know we have been going back and forth with the stability studies with the FDA. As I updated you last time, we had a meaningful discussion with the FDA and as we understand now, they really did not want us to submit all the raw data nd the detailed information. All they were interested in was for us to let them know that the compound 1a(OH)D5 is stable at room temperature. As you know, we showed that it is stable at room temperature for 159 days. However we had done all these previous correspondence according to the advice of our consultant.

Now we have to do the entire stability testing again. However this time, instead of us conducting studies we are having them done professionally. The studies will be done by a company called SaidruSyn. This is India based company, they had synthesized our D5 for clinical studies. They have much experience dealing with the FDA also. So hopefully, we will be able to get approval this time. The capsules have been

prepared for the entire stability studies and will be shipped to India this week. The lack of obtaining approval is clearly beyond our control and actually it has nothing to do with the compound being unstable. It is very stable at room temperature. But the FDA also have their own guidelines they must follow and we certainly received some bad advice on this, which resulted in extended delay in getting approval for the Phase I trial.

I apologize for the delay in intiating studies with prostate cancer but clearly it really depends on our getting approval from the FDA. If you have any specific questions please do not hesitate to contact me.

Thank you very much,

Sincerely,
Rajendra Mehta

Rajendra G. Mehta, PhD
Assistant Vice President and Head
Carcinogenesis and Chemoprevention Division
IIT Research Institute
Professor, Biological Sciences, IIT
10 West 35th Street
Chicago, IL 60616

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e-mail: RMehta@iitri.org

AMENDMENT OF SOLICITATION/MODIFICATION OF CONTRACT

1. CONTRACT ID CODE: S
PAGE OF PAGES: 1 | 2

2. AMENDMENT/MODIFICATION NO.	3. EFFECTIVE DATE	4. REQUISITION/PURCHASE REQ. NO.	5. PROJECT NO.(If applicable)
P00004	09-Mar-2006	FORM96PC010148-000	

6. ISSUED BY CODE W81XWH
USA MED RESEARCH ACQ ACTIVITY
820 CHANDLER ST
FORT DETRICK MD 21702-5014

7. ADMINISTERED BY (If other than item 6) CODE W81XWH
USA MED RESEARCH ACQ ACTIVITY
ATTN:WENDY BAKER
WENDY.BAKER@AMEDD.ARMY.MIL
FORT DETRICK MD 21702-5014

8. NAME AND ADDRESS OF CONTRACTOR (No., Street, County, State and Zip Code)
UNIVERSITY OF CALIFORNIA DAVIS
ONE SHIELDS AVENUE
118 EVERSON HALL
DAVIS CA 95616-8671

CODE 1CBG4 FACILITY CODE

9A. AMENDMENT OF SOLICITATION NO.

9B. DATED (SEE ITEM 11)

X 10A. MOD. OF CONTRACT/ORDER NO.
DAMD17-02-1-0070

X 10B. DATED (SEE ITEM 13)
19-Feb-2002

11. THIS ITEM ONLY APPLIES TO AMENDMENTS OF SOLICITATIONS

☐ The above numbered solicitation is amended as set forth in Item 14. The hour and date specified for receipt of Offer ☐ is extended, ☐ is not extended.

Offer must acknowledge receipt of this amendment prior to the hour and date specified in the solicitation or as amended by one of the following methods:
(a) By completing Items 8 and 15, and returning ______ copies of the amendment; (b) By acknowledging receipt of this amendment on each copy of the offer submitted; or (c) By separate letter or telegram which includes a reference to the solicitation and amendment numbers. FAILURE OF YOUR ACKNOWLEDGMENT TO BE RECEIVED AT THE PLACE DESIGNATED FOR THE RECEIPT OF OFFERS PRIOR TO THE HOUR AND DATE SPECIFIED MAY RESULT IN REJECTION OF YOUR OFFER. If by virtue of this amendment you desire to change an offer already submitted, such change may be made by telegram or letter, provided each telegram or letter makes reference to the solicitation and this amendment, and is received prior to the opening hour and date specified.

12. ACCOUNTING AND APPROPRIATION DATA (If required)

13. THIS ITEM APPLIES ONLY TO MODIFICATIONS OF CONTRACTS/ORDERS.
IT MODIFIES THE CONTRACT/ORDER NO. AS DESCRIBED IN ITEM 14.

☐ A. THIS CHANGE ORDER IS ISSUED PURSUANT TO: (Specify authority) THE CHANGES SET FORTH IN ITEM 14 ARE MADE IN THE CONTRACT ORDER NO. IN ITEM 10A.

☐ B. THE ABOVE NUMBERED CONTRACT/ORDER IS MODIFIED TO REFLECT THE ADMINISTRATIVE CHANGES (such as changes in paying office, appropriation date, etc.) SET FORTH IN ITEM 14, PURSUANT TO THE AUTHORITY OF FAR 43.103(B).

X C. THIS SUPPLEMENTAL AGREEMENT IS ENTERED INTO PURSUANT TO AUTHORITY OF:
Article 16 "Amendment of Grant"

☐ D. OTHER (Specify type of modification and authority)

E. IMPORTANT: Contractor [X] is not, ☐ is required to sign this document and return ______ copies to the issuing office.

14. DESCRIPTION OF AMENDMENT/MODIFICATION (Organized by UCF section headings, including solicitation/contract subject matter where feasible.)
Modification Control Number: w cockerh061462

1. The purpose of this modification is to extend the period of performance to read as show n in this modification. This is being done in accordance w ith the recipient's request dated 8 February 2006. This one-year extension is granted so that the recipient's can obtain the necessary FDA approval of the IND. If the approval is not obtained w ithin the one-year period, the grant w ill receive no further extensions and all unexpended w ill be returned to the Department of Defense.

2. All other terms and conditions remain unchanged.

Except as provided herein, all terms and conditions of the document referenced in Item 9A or 10A, as heretofore changed, remains unchanged and in full force and effect.

15A. NAME AND TITLE OF SIGNER (Type or print)

16A. NAME AND TITLE OF CONTRACTING OFFICER (Type or print)
JOSEPH S. LITTLE / CONTRACTING OFFICER
TEL: 301-619-2546 EMAIL: joseph.little@det.amedd.army.mil

15B. CONTRACTOR/OFFEROR	15C. DATE SIGNED	16B. UNITED STATES OF AMERICA	16C. DATE SIGNED
(Signature of person authorized to sign)		BY Joseph S. Little (Signature of Contracting Officer)	09-Mar-2006

EXCEPTION TO SF 30
APPROVED BY OIRM 11-84

30-105-04

STANDARD FORM 30 (Rev. 10-83)
Prescribed by GSA
FAR (48 CFR) 53.243